FINAL REPORT FOR: "Documenting and predicting the presence of listed vernal pool plants," USDI, Bureau of Reclamation

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Abstract

We developed a new method for surveying rare species in vernal pools, by which investigators focus their search on that zone/microhabitat/plant community within the pool that is known to be associated with the target species. This method, "community-based sampling" or CBS, was tested against two commonly used methods: the whole-pool walk, and transect sampling in which small quadrats are regularly placed along the transects. We used two independent trials to compare the three methods, one that used experts in vernal pool flora and vegetation and one that used untrained university under-graduates. In each trial, we documented the percentage of pools in which the target species was found (accuracy) and the time spent in the search (efficiency). CBS was the most accurate in both trials and—when used by experts—it was also the most efficient. We concluded that CBS had the best performance, producing more accurate results in the least amounts of time. Trials were limited by the funding agency to Merced, Placer, and Sacramento Counties; they took place in late spring and early summer of 2009 and 2010. Our initial list of target taxa was reduced to five by such factors as limited access, the quality of pool vegetation due to the vagaries of precipitation patterns preceding sampling, and the absence of the target at pools for which it had previously been noted: *Castilleja campestris* ssp. *succulenta, Cicendia quadrangularis, Downingia pusilla, Gratiola heterosepala*, *Navarretia myersii* ssp. *myersii, Orcuttia inaequalis,* and *O. viscida. C. quadrangularis* is not a listed taxon, but its low abundance imitated that of rare species, and including it as a target made our trials more complete and robust, especially for claypan pools. The target species collectively included preferential microhabitats ranging from shallow pool edges to deep pool centers, and claypan, hardpan, and volcanic rock pool types.

Introduction

The very nature of listed plant species is rarity of occurrence, often coupled with low abundance and a cryptic habit. In addition, annual vernal pool plants exhibit a wide variation in abundance (and even in presence) from year to year, depending upon that year's weather (Buck 2005). Because the degree of protection afforded vernal pools depends upon the presence/abundance/ecological health of listed plant and animal taxa, it is of utmost importance that agencies with authority over vernal pool ecosystems have a certainty that listed plants are—or are not—present, prior to approving any take and designating appropriate mitigation actions. To maximize certainty, agreement should be reached on the use of sampling designs that optimize accuracy and efficiency.

Sampling approaches based on randomly or regularly chosen sites are of limited value for rare species inventory because it is very unlikely that such sites will contain rare species. Many researchers have been looking for effective ways of taking rare species inventories. The methods they have used are variously called model-based, stratification, multi-response model with the best fit, niche-based model, GIS-based prediction model, and habitat prediction. They all attempt to predict occurrence based on knowledge of the niche requirements of a target species. Some species with small populations are rather common and they constitute a significant proportion of a community's floristic composition. Rare species, in contrast, not only have small populations but those populations are very infrequent. The absence of a rare species can only be determined with confidence by an exhaustive search of the entire habitat, a procedure that is too time-consuming and expensive to be practical.

There is, theoretically, a relationship between the number of vegetation samples taken, the rarity of the target species, and the probability of its detection (McArdle 1990). Guisan et al. (2006) used a niche-based distribution model for predicting the occurrence of endangered species to stratify their sampling design. From both simulation models and actual field observations in Switzerland, they showed their stratified sampling led to a significant improvement of sampling efficiency over simple random sampling. In other ecosystems, a combination of stratification and remote-sensed-based modeling improved the probability of detecting five rare epiphytic macrolichens in the American Pacific Northwest (Edwards et al 2005) and of broadleaved trees in Utah (Zimmerman et al. 2007). Classification and regression tree modeling with augmented with GIS were used to predict suitable/likely habitat for the rare understory herb *Xerophyllum asphodeloides* in pine-oak forests of Virginia (Bourg et al. 2005). Ecological niche modeling helped locate new Indian populations of the critically endangered tree species *Gymnocladus assamicus*, a taxon that had been considered to be extinct (Menon et al. 2010).

The most reliable information about species distributions are in herbaria and museum collections, but this sort of data is only presence-absence. Usage of such data for modeling purposes presents difficulties because there is no parallel set of environmental data for sites where the taxon is absent. Elith and Lethwick (2007) used herbarium records for 226 species from six regions of the world, and applied multivariate adaptive regression splines. They demonstrated that models developed with absences inferred from the set of presence-only sites performed better than models in which pseudo-absences were drawn randomly from the study area. Follow-up research on the effect of pseudo-absence data on the accuracy of distribution models has demonstrated that the choice of background data has as large an effect on accuracy as the choice of modeling method itself (Phillips et al. 2009).

Species distribution modeling uses various statistical methods that combine species occurrence data with environmental spatial data to predict species occurrence. Accuracy of predictions depends upon the quality of data used for refining the model. Graham et al. (2008) analyzed robustness of modeling techniques to location error, and found that certain model techniques (e.g., boosted regression trees and maximum entropy) are particularly robust even in the face of a moderate level of location error. The application of predictive distribution modeling to biological conservation deals with a series of real life problems that can be overcome with the development of systematic baseline datasets, models, and increasing accuracy and availability of remotely sensed ecological data (Rodrigues et al. 2007).

A model-based approach was recently applied to reconstructing suitable habitats for California vernal pool species. Holland and Hollander (2007) used GIS techniques to model the potential distribution of 82 plant and animal taxa associated with vernal pools. They analyzed the relationships between occurrence of taxa with soil, elevation, slope, precipitation, summer humidity, and min/max temperatures in order to identify and map potential habitat for each species, many of which are rare. Their maps have become an important practical tool for planning and conducting rare species surveys.

Objectives

Virtually all of the studies mentioned above attempted to predict the occurrence of rare taxa at the landscape scale. In contrast, our study's objectives focus on predicting rare species occurrence at a very fine, local scale. The basic logic of our approach, however, is very similar: to direct surveyors to a portion of a study area that has the highest probability for containing a given target species. In our case, the study area was a portion of a single vernal pool.

A common protocol currently used for rare species surveys in vernal pools is to make a walking survey of the entire pool, usually on a meandering strip 1-2 m wide, that crosses the pool many times. The advantage of this approach is that the entire pool is examined, but a disadvantage is that the species may occupy only one part of the pool, such as the deepest part of the shallow edge, thus time is wasted searching in unlikely parts of the pool to contain the target taxon. Another common protocol is to sample a number of small quadrats (0.25-1.00 square meters) distributed regularly along transects that cross the pool. The advantage of using this method is that viewing small parts of a pool is easier and probably more accurate than surveying the entire pool; a disadvantage is that only a small fraction of the pool's area is examined and yet the time it takes to establish the grid of quadrats is excessive.

We have added a new sampling protocol that we predict is both more efficient and more accurate in finding target species than the two methods summarized above. We developed the protocol because we hypothesize that efficiency and accuracy will improve if the surveyor first examines those zones/microhabitats/plant communities within a pool that are known to be associated with the target species. We call the method, the "community-based sampling" (CBS). The kind of association knowledge necessary to employ CBS has only recently been accumulated in the course of making a statewide survey of vernal pool vegetation by Barbour et al. (2003, 2005, 2007), Solomeshch et al. (2007).

Our objective is to design a study that will statistically compare the three methods in terms of accuracy and efficiency. The comparisons will also include the possible effect of duripan type, species richness, target growth form, individual surveyor, and category of surveyor (eg, experienced vs novice) in terms of sampling experience and knowledge of the vernal pool flora.

To carry out this study, each participant agreed to take on specific assignments. Dr. Rae served as PI, was the liaison between the research team and the funding agency and UCD, was responsible for handling budget details, and served as the administrator of the project. Drs. Barbour and Solomeshch developed the sampling method protocols, formulated the hypotheses, arranged for the participation of experts and non-experts, and wrote drafts of the report. In addition, Dr. Solomeshch led the field work and data analysis. Dr. Neil Willits directed the creation of the experimental design and determined which statistical techniques to use. We acknowledge and thank the following individuals: Jennifer Buck, Carmella Caria, Robert Holland, Rod Macdonald, John Vollmar, and Carol Witham, who served as vernal pool experts in the field trials; Professor Truman Young and TA Tom Rambo, who inserted a field trip to vernal pools in their course schedule; and finally the students in that class who served as non-experts in the field trials.

Methods

Selection of target species

The funding agency required us to focus on federally listed species in three counties: Merced, Placer, and Sacramento). Our initial list of potential target taxa is summarized in Table 1a. The list was based on CNDDB records, maps created by Dr. Robert Holland, and also on our 10 years of field sampling in the Central Valley (see the last three columns on the right side of Table 1a). Our objective was to choose relatively wide-spread taxa whose distributions and associated plant communities were well-documented. We also chose a group of taxa that included a variety of growth forms and habitats. For example, *Castilleja campestris* ssp. *succulenta,* listed by federal agencies as threatened ("T") and by California agencies as endangered ("E"), is a forb

("forb," a broad-leaved herb) most commonly occurring in communities of deep pools ("deep") underlain by a hardpan ("H"). Its presence has been documented by CNDDB for 91 pools or pool-complexes, and our statewide survey described its occurrence (and later the communities that it was associated with) in 15 separate pools in Merced County, within one of the two vernal pool regions defined by Keeler-Wolf and Evens (2010) that are known to contain *Castelleja campestris* ssp. *succulenta* ("1/2 regions").

During 2009, Dr. Holland and Jennifer Buck rejected four potential target taxa for mixed reasons: they were not seen at candidate sites that particular year, probably because of the vagaries of weather; or they would be difficult to access because of difficult land owners or because of extreme rural locations that would be too time-consuming to reach. The rejected taxa were: *Astragalus tener* var. *tener, Downingia pusilla, Gratiola heterosepala, Lasthenia conjugens, Legenere limosa,* and *Limnanthes floccosa* ssp. *californica* (Table 1A). Sampling in 2009 utilized four targets: *Castilleja campestris* ssp. *succulenta, Navarretia myersii* ssp. *myersii, Orcuttia inaequalis,* and *O. viscida*. Sampling in 2010 utilized one target: *Cicendia quadrangularis*.

Herbarium specimens of these five taxa and of closely related taxa were carefully examined and searched for distinctive differences between the target taxon and similar-looking relatives. The most problematic of the five are summarized in Table 1B.

Castilleja campestris ssp. *succulenta* differs from *C. campestris* ssp. *campestris* in Petal color, anther sac length, leaf shape and texture, and bract shape, texture, and number. *Gratiola heterosepala* differs from *G. ebracteata* in leaf shape, in sepal shape, and length, and in corolla coloration. *Navarretia myersii* ssp. *myersii* differs from *N. leucocephala* and *N. prostrata* in degree of prostration, inflorescence type and branching, length of peduncle, and in corolla length and the ratio of corolla:calyx length. (Another close relative is *N. myersii* ssp. *deminuta,* but it is restricted to Lake County, far from the areal limits of our study.) Drawings and photographs of target taxa and close relatives were made available to field crews, so that they could continuously check themselves.

Selection of surveyors

In 2009, all sampling was done by widely acknowledged vernal pool experts Rod Macdonald, Virginia Meyer, Christy Owens, Ayzik Solomeshch, Steve Talley, John Vollmar, and Carmela Caria. Jennifer Buck and Bob Holland formed a pre-trial team whose task was to find with target species (and one pool without the target species) and to record their GPS coordinates. They did not participate in the trial itself because the extra knowledge they possessed would have biased their sampling. The only exception from the rule was Ichord Ranch, for which the pools were identified by John Vollmar. Once the surveyors were at the site they used pin flags to mark and number the pools to be sampled.

In 2010, all sampling was done by university undergraduates enrolled in a course on the natural vegetation of California. Although some students in the class had taken plant taxonomy or general ecology courses, the average student had only taken an introductory

1-quarter plant biology course and did not have a large knowledge base of the California flora, let alone of the vernal pool flora. Because the trials had to be conducted within the time limits of their class field trip to vernal pools, we were constrained to conduct the trials at Jepson Prairie, a preserve that was protected and managed by UC Davis. Only claypan pools exist at Jepson, and none of the four target taxa from 2009 occur on claypan pools. Therefore, we had to find a surrogate (unlisted) species. Prior to taking the class to Jepson Prairie, one of us surveyed approximately 30 pools, looking for a potential target species that was locally uncommon and difficult to find. *Cicendia quadrangularis* was chosen. It is not a listed taxon nor even a "species of concern" to local conservation NGOs, but its abundance in Jepson pools was low enough to make this species a reasonable surrogate for a formally recognized rare species.

Selection of study sites

Prior to field work, we selected potential field sites by reviewing information about the presence of target taxa from our state-wide survey. Potential sites were then evaluated for ease of physical access, degree of cooperation expected of landowners (based on our past experience with them), availability of aerial photography and soil maps, and our own familiarity with the site—including the certain knowledge that target species were present based on vegetation samples we had taken ourselves, from which we had been able to key out the plant communities present and which ones were associated with the target taxa. The large list of potential sites was narrowed to three: Flying M Ranch and Ichord Ranch in Merced County, and Grand Line Road in Sacramento County. Vernal pools in all three sites were underlined by hardpans and were associated with Riverbank, Laguna, and Mehrten geologic formations on high to low terraces. Soil series included Keyes, Porterville, and Redding. Maps, aerial images, and photographs of target species, vegetation, and surveyors all appear in Figures 1-5. For 2010, we added Jepson Prairie in Solano County (Fig. 6), a large pool complex on claypan, with recent alluvial landforms. Soil series included Alamo. Hardpan soils were 1-2 orders of magnitude older than claypan soils.

Sampling protocols

<u>Whole-pool sampling (WPS).</u> The investigator searches the entire pool, walking along an imaginary path that runs approximately perpendicular to the long axis of the pool, crossing the pool several times (Fig. 7). The investigator walks with a constant speed through pool edge, shallow one, and deep bottom, giving no preference in time or attention to any habitat or visually distinctive vegetation types in the pool. While walking, the investigator focuses the search in a belt approximately 1-2 m wide. The route taken must be possible to complete within the standardized 20-minute sampling time. The time it takes to find the first target plant is recorded, and—if desired—the time it takes to find a second or third plant is also recorded. The time it takes to encounter a target individual a first time is recorded and—if desired—also the time to find a second and third occurrence, the shortness of elapsed time between first and second, second and third, being an indication of a clumped distribution. Although we recorded this information on the data sheets, we later found that the added information was not helpful

in distinguishing among different types and intensities of spatial patterns. Consequently, we have not reported these data in the paper. At the end of 20 minutes, the investigator can decide on a density class to assign the target populations: 1 plant, 2-50 plants, 51-100, 101-500, and >500. Density per square meter can be calculated after the standard 20 minutes by dividing the class's median value by an estimate of the pool's area. The difficulty of training surveyors to reach similar estimates of density, however, was a limitation to the usefulness of these estimates and we have not reported those data in this paper.

Transect-based sampling (BTS). The investigator defines an axis along the longest dimension of the pool and along the shortest (Fig. 7) to be sampled. Those two axes will be the direction of a 1-m-wide belt transect that extends across the entire pool and through its center. The belt can be thought of as a contiguous sequence of 1 x 1 m quadrates. Some fraction of the quadrates (e.g., one-third, one fifth, one tenth) will be searched for the target species, the number of quadrates to be examined being determined by how much time it takes to examine the first two quadrates. For example, if the first two quadrates required 1 minute each, then only 20 quadrates can be examined in 20 minutes, and those 20 will be equally spaced out along the projected route. The time required to find the first target plant is recorded, and—if desired—the time required to find the second and third. As with WPS, after 20 minutes have elapsed, the density class can be chosen and expressed on the basis of pool area in square meters.

Community-based sampling (CBS). The known habitat and plant community preference of the target species are determined from reviewing the literature prior to entering the field. When about to begin sampling a pool, the investigator pauses at the pool's edge to mentally survey the heterogeneity of plant communities that seem to be present in the pool. Based on vegetation physiognomy, color, species composition, and habitat (eg, deep, shallow, edge), the appropriate community type is identified. In some cases, the community type will consist of one large contiguous strip or patch, whereas in others it will be fragmented into several disjunctive, small patches. In the latter situation, the investigator will choose a survey route such that it crosses all patches of that community type within the pool. If the community most likely to contain the target species is an edge strip, the surveyor starts the search clockwise from a starting point he/she defines with a pin flag inserted at the pool edge. If the community consists of one or more patches, each patch is searched by taking a zigzag route 1 m in width (Fig. 7). The time it took to find the first occurrence is recorded and—if desired--the additional time to find the second and third occurrences is recorded. If an individual has not been encountered during the first 15 minutes, sampling will continue in an adjacent community type (in a shallower part of the pool if the search started with a patch in the deepest part of the pool; in an intermediate depth if the search started at the edge). If the initial search had been in an intermediate depth/ zone, then the second search will be in the shallower (edge) direction first and in the deeper direction next. After 20 minutes have expired, the investigator can choose a density class, which can be converted to a per square meter basis, the square meters in this case being only the area containing the associated plant community, rather than the entire pool's area. The time it takes to encounter a target individual a first time is recorded and—if desired—also to the second and third individuals.

Sampling design

We realized that one surveyor cannot apply more than one protocol in a give pool because if he/she has already determined the location of a target species within the pool using one survey type, that knowledge will surely bias the results of a follow-up survey type. Such observations cannot be considered independent and should not be used for any statistical comparison of protocols. To avoid this obstacle in both trials with experts and students each surveyor surveyed every given pool only once using a single search protocol.

For the sake of consistency, each surveyor started the survey from the same point in a given pool, regardless of the protocol they were using. To locate this point, the first person to visit a pool defined the longest possible transect and placed a pin flag at the edge of the pool at the northern end of the transect (Figure 8). If a pool was oriented from west to east, the pin was placed at the eastern end of the transect. If the pool was oriented at some intermediate direction, then the pin was placed at the northeastern or northwestern end of the transect. For transect sampling, the surveyor started the search at this point and continued along the transect. For whole-pool sampling, the surveyor starts the zigzag path to the left of the point (left, when facing the pool). For CBS, the surveyor traveled directly from the starting point to the nearest patch of the community with which the target species was known to associate.

Training and calibration. On 31 March 2009, the vernal pool experts who had agreed to participate gathered at Phoenix Park's vernal pool complexes in Sacramento County for a day of training and discussion, the objective being to learn how to consistently apply the protocols for the three sampling methods and to improve/refine all of them through group discussion (Fig. 9). We selected a surrogate target species which was--on that date--rare and difficult to find, but not one of the four listed target species. Fresh samples of the surrogate species were on hand for examination. Phoenix Park has hardpan pools that tend to be small, a few hundred square meters each. All the trainees had a hands-on experience with all three protocols, and all contributed suggestions for improving the structure of the data sheet (See Appendix), consistency in marking the plots, smoothing the procedures, standardizing the survey routes, and marking the surveyor's start at each pool.

Students were instructed the day of the trial and divided into three groups of nine students each, each group learning how to use only the single protocol assigned to that group. Each student searched three pools and spent 15 min using a single protocol in each pool. (The time was reduced from 20 min to accommodate the limited time given to us by the class's schedule.) The design is outlined in Table 2. Because other students might be close enough to overhear or see where the target species was noted, students were admonished to work quietly and to continue surveying the pool for a full 15 min, even after a first occurrence was recorded. Each student was given a drawing or photograph of the target species and was instructed how to tell the difference between it and closely related taxa that might be in the same pool.

Number of pools sampled. Four pool complexes at three sites were visited by six experts in 2009: Ichord Ranch (*Castilleja campestris* ssp. *succulenta*), Flying M Ranch (*Navarretia myersii* ssp. *myersii* and *Orcuttia inaequalis*), and Grand Line Road (*Orcuttia viscida*). One pool empty of the target was located in each complex, but surveyors were not told how many pools and which were empty, in order to better mimic a real-world monitoring situation. A fifth pool complex was visited by 27 students in 2010: Jepson Prairie (9 pools sampled). Altogether, 29 pools with target taxa were sampled in 2009 and 9 more in 2010.

Data analysis

We estimated two types of accuracy – accuracy of protocols and accuracy of prediction that a target species will occur in a certain plant community. The first was calculated as the percentage of successful surveys in pools that contained target species. This percentage was calculated for each of the three protocols separately. The accuracy of prediction was calculated as percentage of cases when the target species was found in that part of the pool that had been predicted. To calculate this percentage all occurrences of target species detected by all three protocols were combined. All analyses were conducted separately for data derived from expert and non-expert trails.

We started analysis of protocol efficiency applying one-way ANOVA to raw data (that is, minutes spent in the pools up to the time the first target plant was found). Each of the "not found" cases in 2009 was treated as if the target had been found just when 20 min of search time had been reached (15 min for students in 2010). This means that "not found" response was treated like if the species had been found at the last minute of the survey. This doesn't capture the complete picture of what's going on. To minimize the effect of this assumption we used rank-transformed data. To minimize the effect on multiplicative differences between treatments we used log-transformed data.

This initial analysis does not address the possible confounding factors that it may be easier/faster to search for certain target species than others, or that some of the surveyors may be better at searching than others. To account for those factors, a series of mixed-model ANOVA were applied to raw data, log-transformed data, and rank-transformed data. The surveyor was treated as a random effect in these analyses. We tested whether residual errors were normally distributed, a key assumption for this type of analysis. If normality could not be demonstrated, then we used nonparametric tests described later in the Results and Discussion section.

Results and Discussion

Comparison of protocol accuracy

We defined protocol accuracy as the percentage of pools in which the target species was found during a survey. For these calculations only pools in which a target species was known to be present were used. When six experts conducted surveys in 29 pools, target species overall were found in 72% of the cases where TBS was employed, in 79% of the cases where WPS was employed, and in 82% of the cases where CBS was employed. Surveys by students had the same pattern, accuracy being lowest with TBS (48 %), intermediate for WPS (67%), and highest for CBS (78%) (Fig.10).

The effect of protocol on accuracy could not be statistically analyzed because accuracy (percentage of successful surveys) was a single number without repeated measures, hence variance is unknown. However, we consider this estimate reliable because it is based on a large number of surveys. In the experiment involving experts (Fig. 10A), results for all four target species were combined for calculation of protocol accuracy. Only first occurrence of target species was used for this calculation. Six experts conducted surveys in 29 pools, which resulted in 174 observations. Each protocol was used twice in every pool. Consequently the accuracy demonstrated in Figure 10A is based on 58 applications of each protocol. When 27 students conducted surveys, the total number of observations for all three protocols was 81 and the accuracy shown in Figure 10B is based on 27 applications of each protocol.

Comparison of protocol efficiency

We defined protocol efficiency as the average time (in minutes) required finding a target species for the first time. When experts conducted surveys, efficiency was highest (that is, search time was shortest) when the CBS protocol was used and lowest when either the TBS or the WPS protocols were used (Fig. 11A). There was no statistically significant difference between efficiencies for TBS and WPS when raw data were used. CBS averaged 2.7 min shorter—33% shorter—than the mean of TBS and WPS as generated by experts (8.3 min).

When students conducted surveys the mean time they spent in a pool to find target species using TBS, WPS, and CBS was 9.4, 6.8, and 6.4 respectively (Fig. 11B). CBS was significantly more efficient than TBS: on average it took 3.0 min (30%) less to locate target species in a pool. Performance of CBS was a little better than WPS (survey time 0.4 minutes shorter) but not significantly different.

We used one-way ANOVAs to test the significance of differences between protocols. When the ANOVA was run on raw data, the effect of protocol on efficiency--as used by expert surveyors or by student surveyors--was insignificant ($p = 0.166$ and 0.123, respectively). However, since treatment differences are multiplicative rather than additive, we think that log-transformed data are more appropriate. The one-way ANOVA, run on log-transformed data, shows that for experts the community based protocol was significantly better than either of the other two ($p = .0061$), while the non-expert survey still shows an insignificant effect of protocol ($p = .1237$). Residual errors from trials with non-experts are not normally distributed (Table 4), which violates basic requirements for

ANOVA. Taking into account the relatively high value of the Wilk-Shapiro statistic (0.82) and robustness of ANOVA we decided to present these results as preliminary. They should not be interpreted separately but rather in conjunction with results of nonparametric analysis by a Kruskal-Wallis Test and Duncan's Multiple Range Test (Table 5).

The trials with experts were designed to take into account differences in pool size, rare species abundance, and differences among observers. A mixed-model ANOVA was run on the raw data, on log-transformed data, and rank-transformed data. As part of these analyses, we tested whether residual errors were normally distributed, a key assumption of this type of analysis. The value of the Wilk-Shapiro statistic (close to 1.0) indicates that residual errors passed the test of normality. Table 3 summarizes the results of the three analyses in terms of the p-values for each effect. Even the raw data showed significant differences in every attribute. In all but one case the differences among protocols, pool complexes, pools, and protocol*site interaction were significant, whether data had been transformed or not. The insignificance of the protocol*site interaction, using log-transformed data means that the percent improvement in search times when using CBS was similar at all sites.

Residual errors from trials with non-experts did not pass the test of normality (Table 4). These data were then subjected to nonparametric analysis by a Kruskal-Wallis test and Duncan's Multiple range test. Kruskal-Wallis test on rank-transformed data showed significant differences between protocols ($p = .0246$). However, Duncan's Multiple Range Test (Table 5) revealed that only TBS was significantly different, efficiency being significantly poorer (mean rank = 2.63 in a rank scale of 1-3). Although CBS was not significantly different to be a uniquely efficient protocol, average rank (2.07 in a scale of 1-3) was 5% lower in rank than WPS and 21% lower in rank than TBS.

In summary, the overall trend of protocol efficiency was similar in both trials conducted by experts and non-experts. The CBS protocol was significantly better than the other two when applied by experts and was among the best two protocols when applied by students.

Accuracy of prediction

We calculated the number of cases when the target species was found in the predicted plant community. The percentage of those cases was used as a measure of accuracy of prediction. In the experiment by experts all three occurrences of the target species found with CBS, WPS, and TBS were combined for calculation of this percentage. In the trails by experts the target species (all four) were found 351 times, among which the number of times when species occurred in the predicted zone/community was 335, corresponding to 95% accuracy. In the trial by non-experts the target species was found 52 times of which the species occurred in the predicted zone/community 41 times corresponding to 79% (Figure 12).

We can reach several conclusions from the results: (1) Our hypothesis that CBS is more accurate and efficient than TBS or WPS was supported. Although most rare vernal pool species are small and difficult to find, plant communities are relatively large and easily seen. Thus, if a given community is known to be associated with a particular rare taxon, then focusing the search process in a vernal pool to that plant community is the most efficient and accurate approach. (2) CBS is relatively easily learned in the field even by non-specialists, but some preparation prior to going to the field is necessary. Office work will include reviewing those plant communities and their character species (or zones and microhabitats) known to be associated with the target species, and this task may be more effective and definitive with the help of a vernal pool expert, if the monitoring person is relatively untrained in vernal pool vegetation. (3) The CBS protocol was successful in a wide range of habitats and plant communities, including hardpan pools and claypan pools, shallow-to-deep habitats or zones, and in a diversity of target plant growth forms, such as forbs vs. grasses and early-maturing populations vs. late-maturing populations. (4) Future work to extend these conclusions could include sampling in volcanic pools and in counties outside of Merced, Placer, and Sacramento that are in other vernal pool regions of the state and support listed taxa other than the four we used. (5) Of course, there are caveats and limitations to employing the CBS protocol for all target species. For example, some rare taxa are not strongly and significantly associated with only one plant community, but instead are weakly associated with several communities. It's possible that a given vernal pool might contain more than one community type that is associated with the target, making the search more complex and time-consuming.

Planning considerations and recommendations

The most cost-effective survey method for sensitive species in vernal pools is community-based (CBS), meaning that surveyors must review the recent literature that has been building in California over the past decade. They must be trained in the method, because being proficient with CBS will require having a larger grasp of the vernal pool flora than most monitoring staff or consultants typically have. This knowledge is important for finding in the field particular community types. Further work is also required in completing the classification of vernal pool community types, in particular outside the Central Valley, because the CBS protocol depends on a thorough background of information (a complete database) of community types and sensitive plants. The statewide survey will be completed and published if modest grant support can be received from state and federal agencies.

Nevertheless, this report identifies the importance of identifying target species within the area to be surveyed prior to survey, together with their diagnostic characteristics and habitat preferences. Based on an understanding of listed plant habitat preferences, the survey protocols should be based on an expectation where the target taxa may be best found. The probability of finding the target species in preferred habitats being higher than finding them in habitats where they had not previously been found, fitting the protocol to the distribution of the preferred habitat thus streamlines the field effort.

Identifying the preferred survey protocol is based on the distribution of the known preferred habitat within the area to be surveyed. The agency program manager as well as the field surveyor should understand relationship between the target taxon and the preferred habitat, as well as the distribution of such habitat, in order to develop an appropriate and cost-effective protocol. And, such a protocol is linked to the site in question and may not translate well to another site with different habitat characteristics.

Noting that the survey effort conducted by students produced results equally successful to the effort by experienced professional botanists suggests that a well-crafted survey protocol may obviate the need for employing only professional botanists in listed plant surveys. Survey design and field supervision by professional botanist should provide rigor equal to that involved in this effort, thus yielding valid results.

The finding that survey protocols emphasizing efforts on preferred habitats should be more effective than random survey protocols should extend to other non-vernal pool sites, but should be tested.

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TABLES AND FIGURES

Table 1A. Initial and final taxa chosen for sampling. The names of the final four are **bold-face**. Listings are US first, CA next, and CNPS third. Functional type relates pool position (shallow, deep, intermediate), grass or forb, and type of substrate (H for hardpan, C for claypan, R for volcanic rock). CNDDB sites is the total record of locations for each listed taxon according to data stored with the California Department of Fish and Game. Pools and regions are from our state-wide survey: how many pools sampled for plant community types showed the presence of a given rare taxon, and how many of the state's vernal pool regions known to support that taxon were included in our survey. The last column lists the county in which we concentrated the work for this paper, and thus only pertains to the final four taxa. The forb *Cicendia quadrangularis* was used at Jepson; it occurs at intermediate depths in H sites but is not a listed taxon, so it's absent below.

Taxon	Listing by US/CA/CNPS	Functional type	CNDDB sites	State-wide survey Pools	State-wide survey Regions	County focus
Astragalus tener var *tener*	-/-/1B.2	shallow, forb, C	67	10	2 / 5	-
Castilleja campestris ssp ***succulenta***	T/E/1B.2	deep, forb, H and R	91	15	1 / 2	Merced
Downingia pusilla	-/-/1B.2	deep, forb, C and H	124	16	4 / 6	
Gratiola heterosepala	-/E/1B.2	deep, forb, H and R	87	9	5 / 6	
Lasthenia conjugens	E/-/1B.1	shallow, forb, C	32	11	2 / 2	
Legenere limosa	-/-/1B.1	deep, forb, H and C	61	9	3 / 8	
Limnanthes floccosa ssp. *californica*	E/E/1B.1	shallow, forb, H	16	2	1 / 1	
Navarretia myersii ssp. ***myersii***	-/-/1B.1	shallow, forb, H	14	24	1 / 2	Merced
Orcuttia inaequalis	T/E/1B.1	shallow, grass, H	52	1	1 / l	Merced
O. viscida	E/E/1B.1	shallow, grass, H	20	6	1 / 1	Sacramento

Table 1B. Diagnostic characteristics of target species

Castilleja campestris* ssp. *succulenta

	C. campestris* ssp. *succulenta	***Castilleja campestris* ssp. *campestris***
leaves	lanceolate, thick, brittle like	linear, thin, flexible
bracts	lanceolate , thick, brittle like, > flowers	linear, thin, flexiable, < or = flowers
corolla	deep yellow to orange	light to bright yellow
anthers	lower anther sac = 1/2 upper	lower anther sac 1/4 - 1/3 upper

Gratiola heterosepala

	Gratiola heterosepala	***Gratiola ebracteata***
leaves	Leaves and sepals truncate	Leaves and sepals long tapered
bracts	Sepals 4-6 mm, unequal	Sepals 8-11 mm, equal
corolla	Corolla yellow (only 3 lower lobes white)	Corolla throat yellow, limb white

Navarretia myersii* ssp. *myersii*

	Navarretia myersii* ssp. *myersii	***Navarretia leucocephala***	***Navarretia prostrata***
plant	prostrate	generally not prostrate	Prostrate
inflorescens	head, not branched	dense cyme with conspicuous branches	head, not branched
flowers	sessile	subsessile or short-peduncled	Sessile
corolla	12-21 mm, tube thread-like 2-4 times longer than calyx, long-exerted	4-10 mm, tube slightly exerted from calyx	7-9 mm, tube slightly exerted from calyx

* The closest relative of *Navarretia myersii* ssp. *myersii* is *N. myersii* ssp. *deminuta*, which was recently (1992) described and currently known only from one type location in Lake County far away from Merced County where we conducted our survey. Morphologically it differs from *N. myersii* ssp. *myersii* in heaving a blue, shorter corolla (12-13 mm). Corolla tube of *N. myersii* ssp. *deminuta* is about the same length as calyx.

Table 2. Design of trial at Jepson Prairie, which included 9 pools and 27 student surveyors. Three students, each using a different protocol, sampled each pool. Protocol T = TBS, W = WPS, and C = CBS. For example, student surveyor No. 1 searches for the target species in pools 1, 2, and 3, using protocol TBS; surveyor No. 2 searches the same pools using protocol WPS; surveyors No. 4 and 7 replicate surveyor No. 1, meaning that pools 1, 2, and 3 ultimately will have each been surveyed three times by each protocol.

Surveyor No.	Protocol	Pool numbers
1	T	1, 2, 3
2	W	1, 2, 3
3	C	1, 2, 3
4	T	1, 2, 3
5	W	1, 2, 3
6	C	1, 2, 3
7	T	1, 2, 3
8	W	1, 2, 3
9	C	1, 2, 3
10	T	4, 5, 6
11	W	4, 5, 6
12	C	4, 5, 6
13	T	4, 5, 6
14	W	4, 5, 6
15	C	4, 5, 6
16	T	4, 5, 6
17	W	4, 5, 6
18	C	4, 5, 6
19	T	7, 8, 9
20	W	7, 8, 9
21	C	7, 8, 9
22	T	7, 8, 9
23	W	7, 8, 9
24	C	7, 8, 9
25	T	7, 8, 9
26	W	7, 8, 9
27	C	7, 8, 9

Table 3. Based on mixed-model ANOVAs, there were significant differences among the three protocols, sites (pool complexes), protocol*site interactions, and pools. Only protocol*site interaction on log-transformed data failed to show significance at p <.05. Site = pool complex.

Factor	Raw data	Log-transformed	Rank-transformed
Protocol	0.019	< 0.0001	0.00005
Site	< 0.0001	< 0.0001	< 0.0001
Protocol*Site	0.036	< 0.103	0.0005
Pool*Site	< 0.0001	< 0.0001	< 0.0001
Normality	< 0.0001	0.018	NA
Wilk test	W = 0.96	W = 0.98	NA

Table 4. Tests of normality on data generated by non-experts, all showing significant departures from normality. The hypothesis tested is that the distributions are non-normal, thus low p values support the hypothesis of non-normality.

Test	Statistic and value	p values
Wilk-Shapiro	W = 0.821	0.0003
Kolmogorov-Smirnov	D = 0.233	< 0.0100
Cramer-von Mises	W-Sq = 0.262	< 0.005
Anderson-Darling	A-Sq = 1.745	< 0.005

Table 5. Duncan’s Multiple Range test of data from non-experts, on differences in efficiencies of three protocols. Means with the same letter in Duncan Grouping column are not significantly different.

Protocol	Mean	N	Statistical grouping
TBS	2.63	27	A
WPS	2.18	27	B
CBS	2.63	27	B

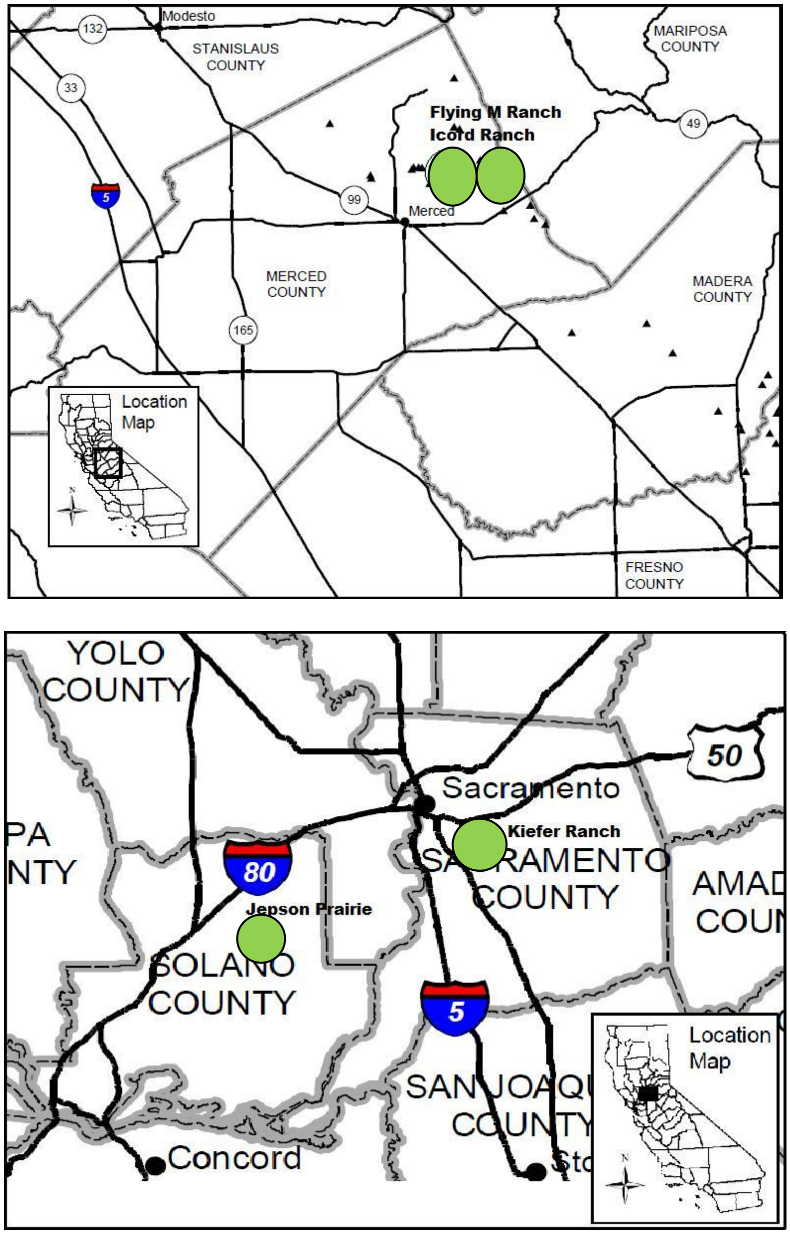

Figure 1. Location of pools in four study areas. (Above) Merced County pools with *Navarretia myersii* ssp. *myersii* and *Orcuttia inaequalis* at Flying M Ranch (left circle). Merced County pools with *Castilleja campestris* ssp. *succulenta* at Ichord Ranch (right circle) (Below) Sacramento County pools with *Orcuttia viscida* at Kiefer Ranch and Solano County pools with *Cicendia quadrangularis* at Jepson Prairie.

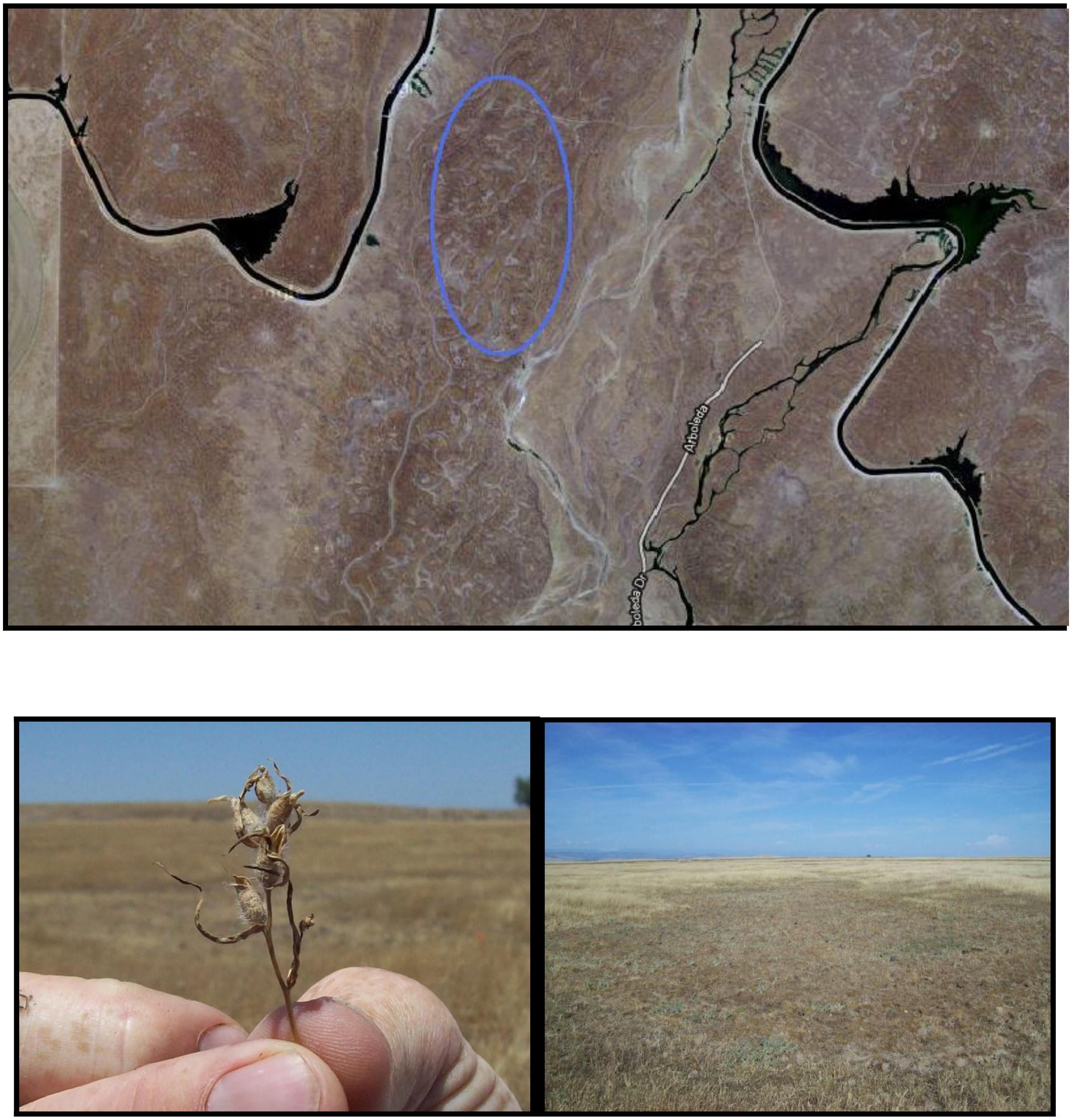

Figure 2. *Castilleja campestris* ssp. *succulenta*. (Upper) Location of pools with *C. campestris* ssp. *succulenta* (within the oval) at Ichord Ranch. (Lower left) Senescent individual plant. (Lower right) Aspect of dry pool when *C. campestris* ssp. *succulenta* was senescent.

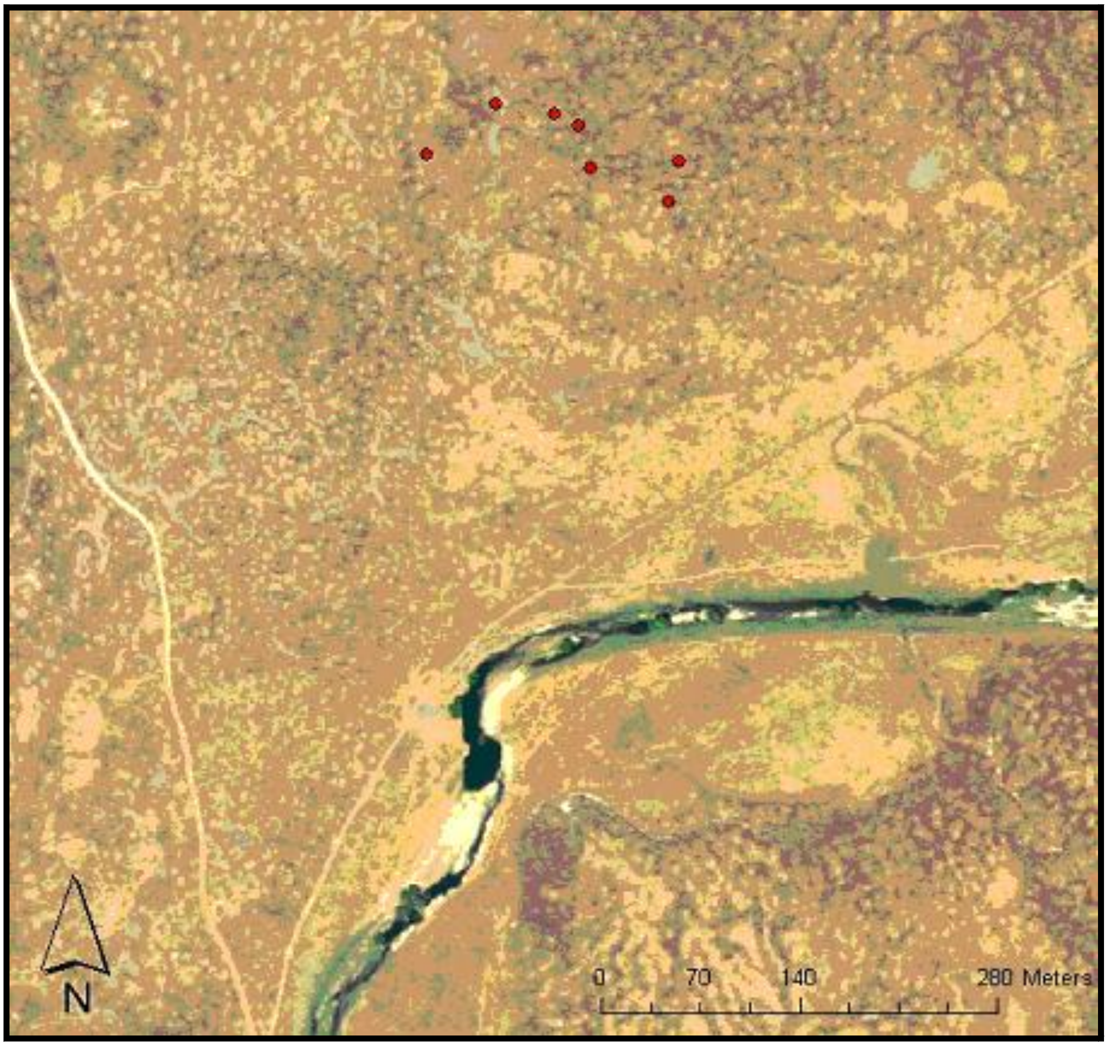

Figure 3. *Navarretia myersii* ssp. *myersii*. (A) Locations (small circles) of seven pools with *N. myersii* ssp. *myersii* in northeastern part of Flying M Ranch. (B) Senescent plants of *N. myersii* ssp. *myersii* at time of sampling, when the target was not easily seen. (C) Aspect of dry vernal pool when *N. myersii* ssp. *myersii* was senescent.

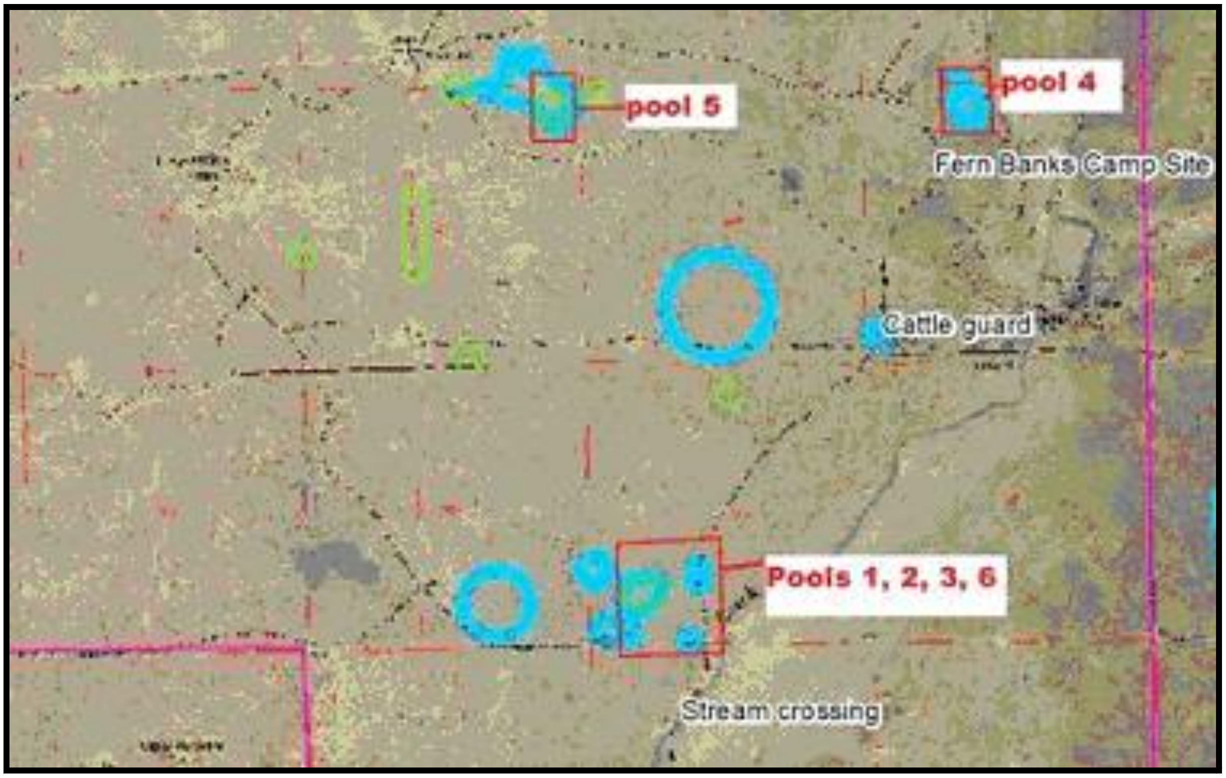

Figure 4. *Orcuttia inaequalis*. (Above) Location of pools at Flying M Ranch with *O. inaequalis*. (Bottom left) *O. inaequalis* was green and in anthesis late in the growing season while most other species were senescent; nevertheless it could be confused with *Crypsis schoenoides*, so sampling was slow. (Bottom right) Aspect of dry pool when *O. inaequalis* was sampled.

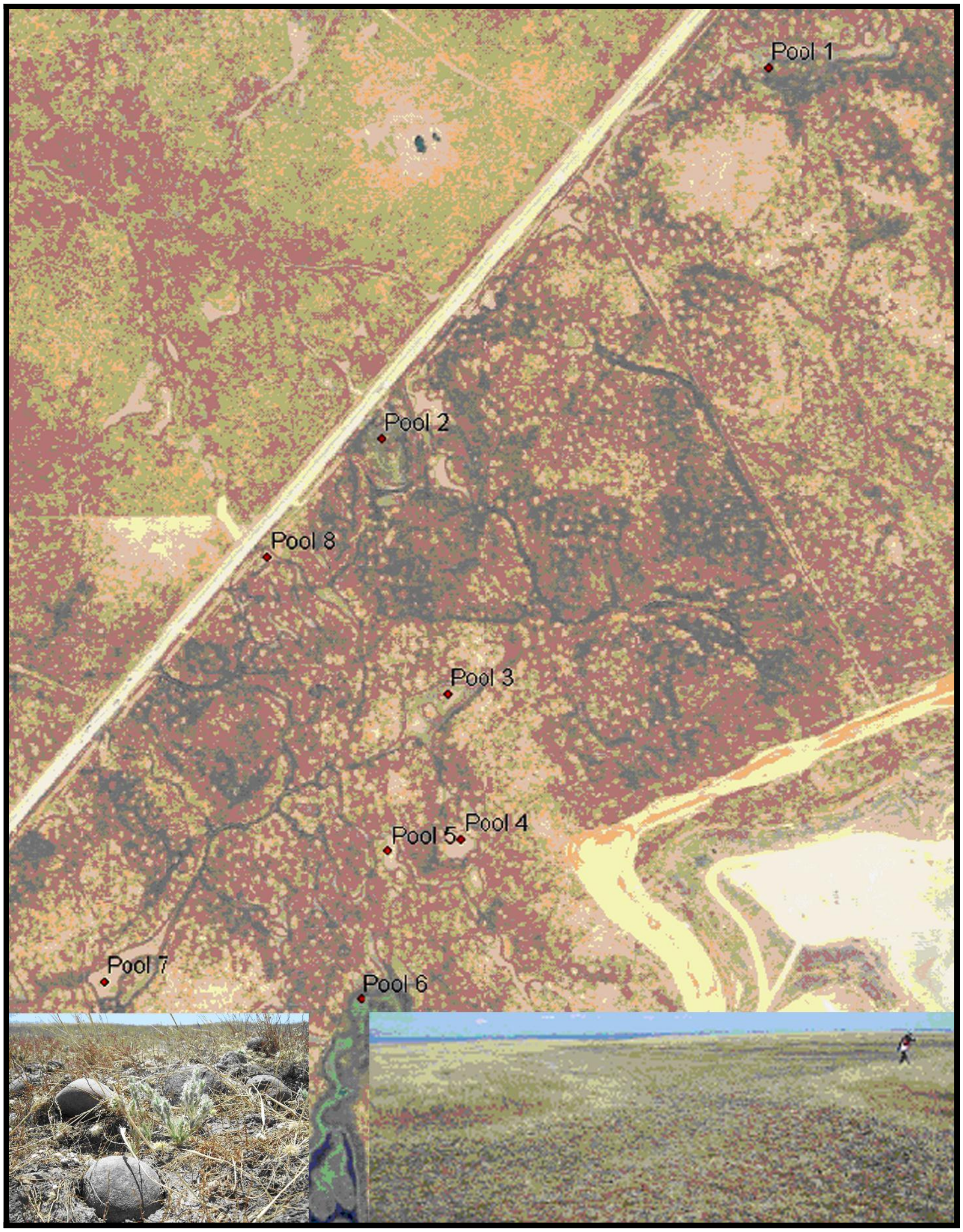

Figure 5. *Orcuttia viscida* at Kiefer Ranch. Aerial view of location of seven pools with *O. viscida* (small diamond symbols). (Inset – lower left) *O. viscida* is a late-blooming species, consequently even small but still-green plants were easily seen against a gray backdrop of senescent vegetation when sampled in late spring. (Inset – lower right) Aspect of vernal pool No. 1 at the time of sampling.

Figure 6A. (Above) Location (circle) of pools with *Cicendia. quadrangularis* at Jepson Prairie. (Below left Typical condition of vernal pool vegetation at the time of sampling in early April of 2010. (Below right) *Cicendia guadrangularis* was not easy to find among other herbs at the time of sampling. The arrow indicates individuals of *C. quandrangularis*.

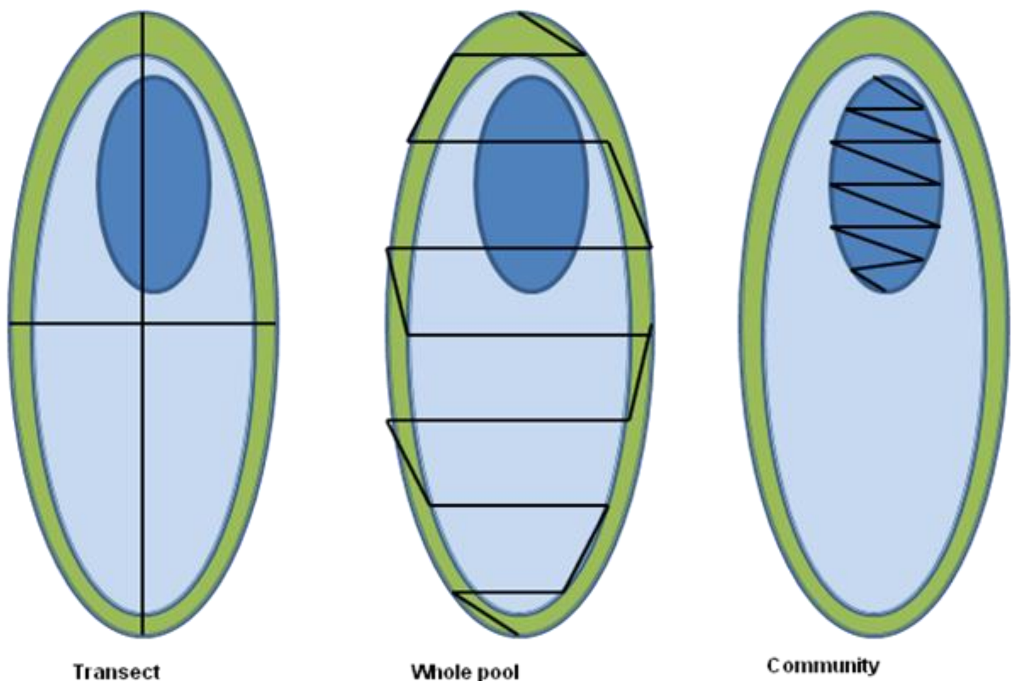

Figure 7. Sampling routes for the belt transect with regularly spaced quadrats along two axes (left), for whole pool sampling (middle), and for the community-based sampling protocol (right) in the case where the target plant community was a deep pool type. The dark narrow strip along the edge represents a plant community restricted to the shallowest part of the pool, the lightly shaded portion represents a second plant community type restricted to moderate depths of standing water, and the dark patch inside both the edge and the intermediate depth zone represents a third plant community type restricted to the deepest part of the pool. Quadrates are not shown on the belt transect diagram.

Figure 8. Marking pools prior to sampling. (Upper) Pool number 5 at Flying M Ranch; at each site, 6-12 pools were marked and numbered with spray paint. (Lower) Ichord Ranch, pool number 11; each surveyor started his/her survey at the same point regardless of protocol being used. The first person to survey a given pool generally defined (by observation) the longest axis through the pool and planted a pin flag at the northern terminus of the axis (at the point where the axis met the pool's edge).

Figure 9. Portion of the research and field teams discussing survey protocols at a 2009 training session in Phoenix Park, Sacramento County. Present were: Michael Barbour, Jennifer Buck, Carmela Caria, Robert Holland, Rod Macdonald, Christy Owens, Steven Talley, and John Vollmar. Photo taken by Ayzik Solomeshch.

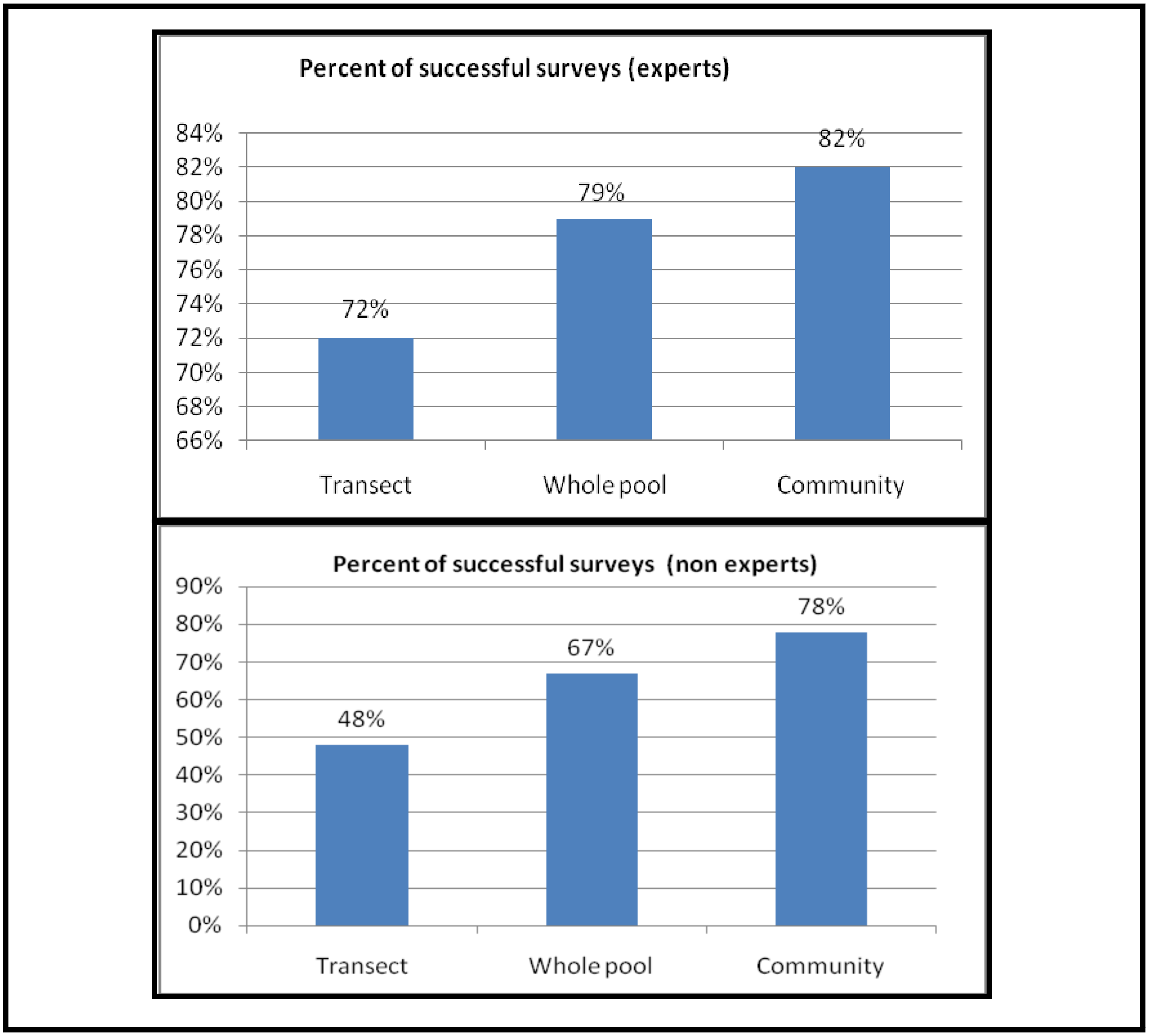

Figure 10. Accuracy of protocols when experts conducted the trials (Upper) and when students conducted the trials (Lower). The pattern was the same for both groups, TBS having the lowest accuracy and CBS the highest accuracy.

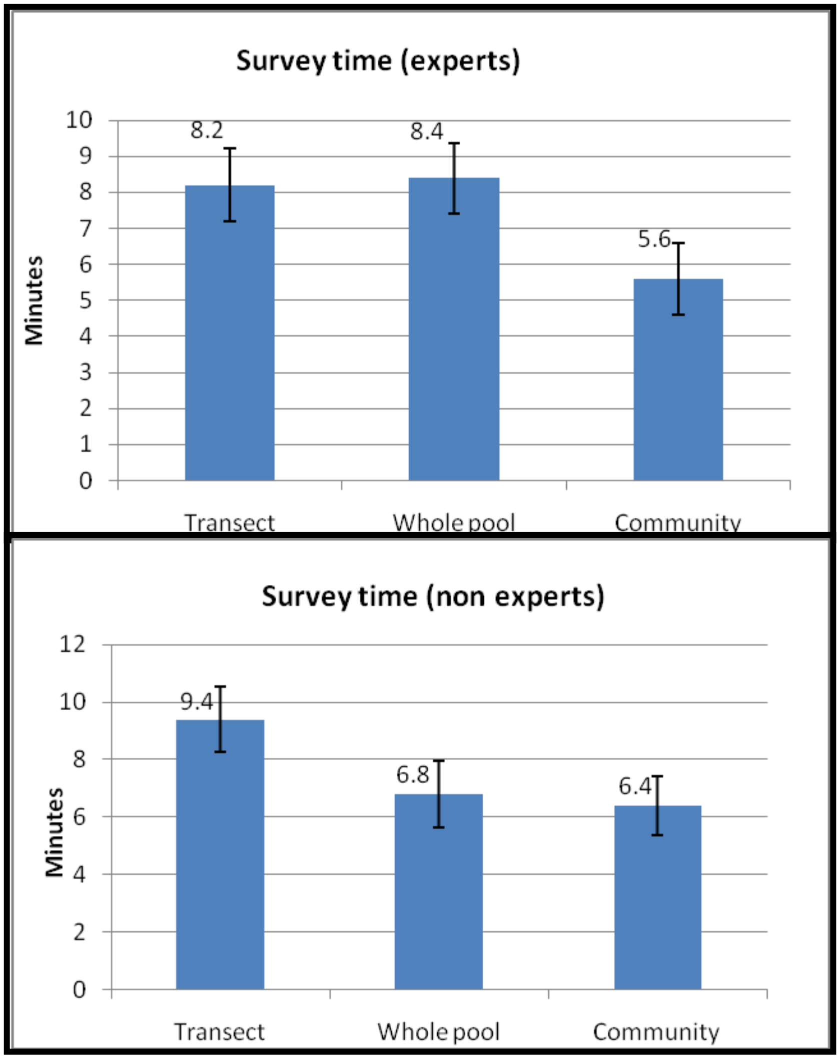

Figure 11. Efficiency of protocols shown on raw (not transformed) data. (Upper) When experts conducted surveys, efficiency was highest (that is, search time was shortest) when the CBS protocol was used. There was no statistically significant difference between efficiencies for TBS and WPS. (Lower) When non-experts conducted surveys, efficiency of CBS was higher than TBS but it was not significantly different from WPS. In the trial involving experts (Upper), survey time for all four target species are combined in this chart.

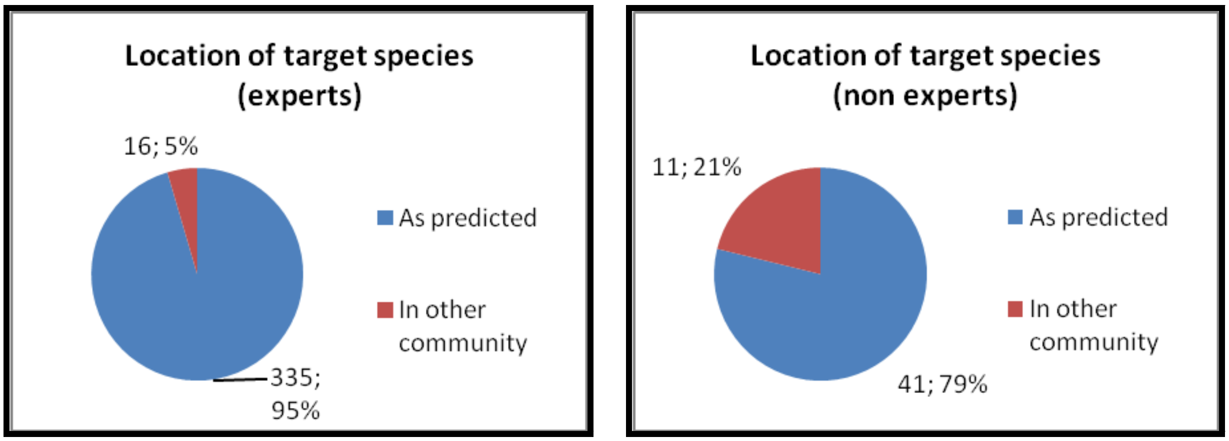

Figure 12. Accuracy of prediction of target species location was 95% in the survey by experts (Left) and 79% in the non expert survey (Right).